Croonian Lecture: *The Respiratory Process in Muscle and the Nature of Muscular Motion.*

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Mr. President and Fellows:—We are keenly sensible of the honour done to us in our being called to lecture on this occasion, and in making this acknowledgment we would express our special gratification in being so enabled to pay this act of piety to the memory of William Croone, whom we commemorate to-day. The Croonian Lecture was founded through his generosity in order to encourage the study of muscular motion, but some sixteen years have now passed since that subject was last treated by the Lecturer. During those years many additions have been made to our knowledge of the subject, and great changes have resulted in our views of it. It is a pleasure to us that we have now the opportunity of taking up again the broken thread of the series, and of turning to-day to the chosen subject of Croone's own enquiries and chief interest. We could wish that a time more free from other occupations and anxieties than the present had allowed us to do this less unworthily.

Croone found in muscle the chief immediate hope of studying the energy discharges of living elements, and it was surely an enlightened instinct which led him to foresee, however dimly then, what we must recognise as still true after this lapse of two and a half centuries. We still must look to the study of muscular motion as the most fruitful, and perhaps for some time to come the only, avenue to intimate knowledge of the modes of energy discharge by the living cell, and of their relation to the specific chemical processes of life. More than this, it is the study of muscle activity which has so far given us all we know of the meaning of respiration as the accompaniment of life. The study of respiratory exchanges in the lungs and in the blood of mammals has given us valuable lessons, and has unfolded attractive stories of animal adaptation to environment. That study takes its place in the natural history of the Vertebrates, and has a living value for the purposes of human medicine. It is describing to us the modes in which oxygen reaches and carbon dioxide leaves the cell under the anatomical conditions of the vertebrate animal, but it does not attack the intimate problems of respiration as a process of animal cell life in general. Croone, of Cambridge, was too close in time and sympathy to the genius of Mayow, and to the work of his other contemporaries at

Oxford, not to realise that in the study of muscle lay probably the first path to knowledge of the inner processes of life within the living substance itself.

Intramolecular Oxygen and the Theory of "Inogen."

The closing years of the nineteenth century, and with them the last occasion on which the Croonian Lecturer dealt with the subject prescribed by the endowment, marked what seemed the final establishment of a particular conception of respiration in muscle. It was almost universally held that muscular energy and, by inference, the energy liberated in any cell upon activity, whether as mechanical energy or heat, sprang from a more or less explosive splitting of a molecular complex which had been made highly unstable, that is "irritable," by the previous inclusion within it of oxygen taken in by the cell during rest. The breakdown of this hypothetical molecule was supposed to yield both lactic acid and carbon dioxide, these being the two obvious and recognisable products of activity.

The earliest phases of this conception, through all the stages of the long-delayed discovery of oxygen, connected the idea of "irritability" directly with that of combustibility. This notion, however, was negatived by the discovery of Spallanzani that living tissues could long survive and continue to yield carbon dioxide without any supply of oxygen except such as had previously been available. This yield of carbon dioxide in the contemporary absence of oxygen was shown to be true for the case of isolated muscle by Müller, Liebig, Matteuci, and others, and it was upon this observation that Hermann chiefly based his theory of inogen. He showed in 1867 that free oxygen was not present in the air pumped from isolated frog's muscle, and yet he found that without any fresh oxygen supply from outside, carbon dioxide was yielded by the muscle when it contracted or when it stiffened after death. At the same time, lactic acid was produced, as had previously been shown, while no nitrogen bodies could be recognised as appearing.

Hermann's hypothetical "inogen" accordingly was the unstable precursor of both lactic acid and carbon dioxide, a precursor in which oxygen was already combined, or placed in a position to combine, with carbon and hydrogen in the combustion which was to yield the energy of contraction. After the explosive breakdown of this precursor, it was supposed that fresh carbon bodies, and perhaps also the lactic acid, were combined again in a newly oxygenated unstable molecule of inogen.

This inogen hypothesis of Hermann was taken up again ten years later by Pflüger in his well known studies of "physiological combustion." Here they were amplified and illustrated with great wealth of rhetoric, but without significant change and without fresh experimental support. Pflüger's

"giant" molecule, as he described it, made unstable by the inclusion within it of what he called "intramolecular oxygen," was the same in all essentials as the inogen molecule of Hermann.

These conceptions of Hermann and Pflüger have had an historical importance reaching far beyond the particular enquiry into muscular energy. They summarised the only aspects of cell metabolism which had received any experimental analysis at all, and up to the end of the nineteenth century they not only represented all that was known of cell respiration and of its relations to cell energy, but they dominated also all our ideas of cell metabolism in general. It was conceived that the chemical processes of life in all cells consisted essentially in the building up of elaborate, unstable, and oxygen-charged molecules, by the processes of so-called "anabolism," into the mystical complexes of irritable protoplasm. From protoplasm, as seen in chemical imagination, a descent by the stages of so-called "catabolism" was conceived to follow, by which through successive splitting processes energy was discharged, and certain recognisable end-products were displayed.

Michael Foster, a name familiar and loved in this place no less than in Cambridge, wrote in 1895 as follows:—

"The oxygen taken in by the muscle, whatever be its exact condition immediately upon its entrance to the muscular substance, in the phase which has been called 'intramolecular,' sooner or later enters into a combination, or, perhaps we should rather say, enters into a series of combinations. We have previously urged that all living substance may be regarded as incessantly undergoing changes of a double kind, changes of building up, and changes of breaking down. . . . We cannot as yet trace out the steps taken by the oxygen from the moment it slips from the blood into the muscular substance to the moment when it issues united with carbon as carbonic acid. The whole mystery of life lies hidden in the story of that progress, and for the present we must be content with simply knowing the beginning and the end."*

The story of that progress is part of the story we have to tell to-day, and these words of Foster may be taken as the summary of what was the current physiological opinion some eighteen years ago, when the work now to be discussed began at Cambridge.

We must note first that the inogen theory had two main bases of experimental support. These were:—

(1) The contraction of muscle and the death of muscle alike were believed

* 'Text Book of Physiology' (Sixth Edition), Book II, p. 610.

to give a simultaneous fresh production of lactic acid and of carbon dioxide in the absence of an immediate oxygen supply. The introduction of oxygen and the preparation for combustion had taken place beforehand.

(2) The instability of the inogen molecule increased with rise of temperature, and so also the rate of production of lactic acid and carbon dioxide, but it was believed that, if the muscle were scalded suddenly with boiling water, the molecule could be "fixed" without a yield either of lactic acid or of carbon dioxide.

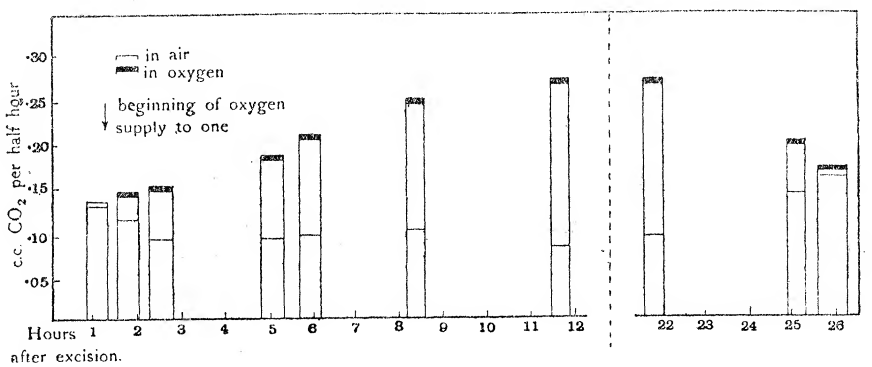
The evidence under both these heads was first examined by one of us so far as the carbon dioxide production was concerned (1).

It will be enough now to recall that in this work improved titration methods were used for the estimation of carbon dioxide, in place of Hermann's eudiometric method, and that the new knowledge of bacterial action allowed the results of early putrefaction to be recognised and left out of account.

By successive estimations, the course of carbon dioxide discharge from isolated frog's muscle was followed. At rest the muscle gave a high initial rate of discharge, which soon descended to a lower rate, maintained at steady level for many hours.

When the muscle was stimulated to contract, an outburst of carbon dioxide such as Hermann had found, and as all the text-books of the day described, was expected and looked for, but none was found; no increase of carbon dioxide accompanied contraction unless the contraction was forced by repeated strong stimulations to give marked fatigue.

At rest again, the muscle in nitrogen gave a lower steady output of carbon dioxide than in air; in oxygen it yielded carbon dioxide two or three times as fast (fig. 1).



1.—Survival discharge from "crossed" pairs of legs, one in air, the other in oxygen. From the 'Journal of Physiology,' vol. 28, p. 354 (1902).

On contraction in oxygen, and now even on slight contraction, it yielded the increase of carbon dioxide expected by the text-books to be shown in air [(3) and fig. 2.]

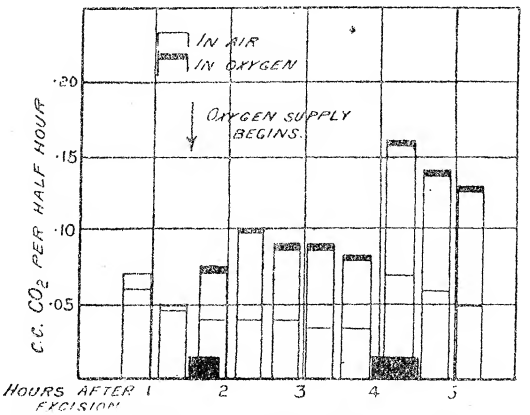


Fig. 2.—Course of survival discharge of carbon dioxide from two "crossed" pairs of gastrocnemii. Contraction periods are shaded. Temp. 17° C. (In the first contraction period slow rhythmic stimuli were given and fatigue was not shown; in the second period, rapid stimuli were given and the muscles were fatigued to a standstill.) For details see the 'Journal of Physiology,' vol. 28, p. 474 (1902).

It may be said in passing that these results accorded with many observations made previously upon the whole animal in which conditions of imperfect oxygen supply had given apparent incompletion of oxidative processes, and explained many earlier discordant observations made when blood was circulated through muscle.

It became clear then that the contemporary and immediate supply of oxygen did affect the products due to contraction, and the inogen theory, postulating a previous inclusion of oxygen within the muscle elements, was evidently inadequate.

We now come to the second of the two pillars of the inogen hypothesis—the effects of heat upon muscle and the supposed "fixation" of inogen by rapid scalding. The close historical dependence of the hypothesis upon the experimental results of heating has not, we think, been sufficiently recognised.

Du Bois Reymond had made the observation that a muscle if slowly killed by heat became markedly acid, but not if it was rapidly killed by scalding. Hermann, in his view of inogen, assumed that lactic acid and carbon dioxide found in it their common and simultaneous source of origin, and, probably biassed by this, he claimed to show experimentally that scalded muscle yielded not only no lactic acid, but also no carbon dioxide; but in fact, though this simply tested phenomenon became the commonplace of the text-

book and has so remained almost to this day, the evidence given by Hermann's eudiometric experiments cannot be accepted on examination.

It was long ago shown at Cambridge (1) that a large volume of carbon dioxide is expelled from the muscle if rapidly scalded, though it was shown also later (5) that, as du Bois Reymond had found, practically no lactic acid is produced.

More recently it has been shown (17) that there are two sources of the carbon dioxide expelled on heating; one is the preformed carbon dioxide held probably in union as carbonate, which is displaceable by acid but not by heat, while the other is that held in firmer chemical union with protein groups, not displaceable by acid yet dissociable near the boiling temperature. On slow heating, with consequent acid formation, only the former source yields carbon dioxide; on rapid heating, without acid formation, and at the high temperatures (80—100° C.) necessary if heating is to be rapid enough, the firmly held carbon dioxide, and that only, is dissipated. Heating, as such, though it may produce a maximum yield of lactic acid, is not accompanied by any fresh production of carbon dioxide.

Pflüger (with Stintzing) also supported the origin of carbon dioxide from a previously oxygenated "giant" molecule by finding that after washing with acid to expel any previously formed carbon dioxide, the giant or inogen molecule broke down on heating to give what they believed to be, and called, "newly engendered carbonic acid." There were grave fallacies however in their technique, and, putting the matter as shortly as possible, when the trial was repeated at Cambridge by better methods it was found that acid applied to muscle at 0° C. expels the preformed carbon dioxide, that the muscle thereafter raised to 40° C. (when the maximal lactic acid yield is given) gives now no carbon dioxide, while heating further to 100° C. gives the normal amount for that temperature as from an untreated muscle (17). Pflüger had in fact been misled by his failure to recognise the double mode of storage of carbon dioxide in muscle. His "newly engendered" carbon dioxide was the carbon dioxide dissociable from muscle proteins on their coagulation at high temperatures, and it has no relation to the energy store of muscle.

The last historical support of the inogen theory then, that of the results of heating, breaks down. So far from lactic acid and carbon dioxide arising together from a common precursor, as Hermann and Pflüger taught, we see that only the particular conditions of experiment determine whether a carbon dioxide discharge appears to accompany lactic acid formation in the muscle or not.

In a muscle at rest in air, and more rapidly in a muscle in nitrogen, lactic

acid is continuously produced (5) and proportionate volumes of preformed carbon dioxide are expelled; as we saw earlier, the slow yield of carbon dioxide is steadily maintained for many hours from an isolated muscle (1). On contraction, and especially in nitrogen, acid production is faster, and the carbon dioxide is expelled faster (3). So we return to the ancient observation of Spallanzani that carbon dioxide is yielded (as he thought, freshly produced) without the immediate presence of oxygen, and, as we have seen, it was largely upon this supposed evidence of a previously oxygenated precursor of carbon dioxide in the muscle that the inogen theory was later to be erected.

The Effects of Oxygen upon Muscle.

According to the ancient view that the irritability and activity of a muscle depended upon its combustibility, oxygen would be expected to hasten the energy discharge by muscle, and so to act as a stimulant or irritant, very much as oxygen kindles glowing tinder to a flame. But on the inogen hypothesis as developed by Hermann, while oxygen should restore and maintain the capacity for energy discharge by completion of the oxygenation of the inogen molecule, it would not be expected to cause or to favour the explosive splitting of the molecule.

Humboldt in 1795, and many others after, had shown that isolated muscle maintained its irritability longer with an abundant oxygen supply than without, and was longer preserved from fatigue after stimulation. Hermann spoke with uncertain voice on this fundamental point. He claimed that oxygen was irritant and destructive at the surface of a muscle, hastening death, but that in bulky muscles exposed to it, it had a preservative action, maintaining irritability below the surface layers. But he used faulty methods, gave few actual data, and obtained obscure results.

With more appropriate methods it was found at Cambridge (2), as we have seen already, that in oxygen the carbon dioxide yield of the muscle was increased threefold or more (see fig. 1) and that, nevertheless, in spite of this increased combustion the irritability, as many from Humboldt to Joteyko had previously found, was not more quickly exhausted but longer maintained. All irritant gases increase the yield of carbon dioxide by quickening the production of the lactic acid, which expels preformed carbon dioxide held in the muscle. But oxygen, while it would set a combustion flaring, not only delays the stiffening of the muscle, but may altogether inhibit its onset. A muscle forced by stimulation to stiffening may be recalled again by oxygen to its previous flaccidity (3).

This seems to us to be a crucial experiment manifesting an immediate

oxidative removal of some product of activity which is a basis of fatigue and of stiffening, giving at the same time a yield of carbon dioxide as the obvious sign of a completed combustion.

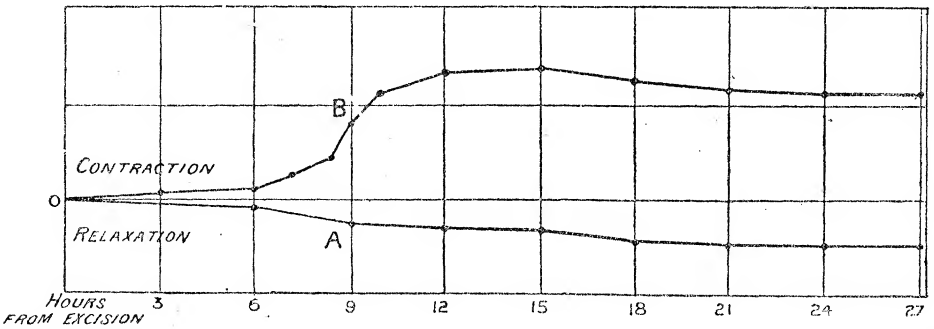


FIG. 3.—Changes in length of a pair of excised gastrocnemii, after fatigue. The ordinates are measured directly from the record upon the drum. The levers magnified $6\frac{1}{2}$ times. Load 3 grm. Temp. 23° C. A. Exposed to oxygen. B. Exposed to air.

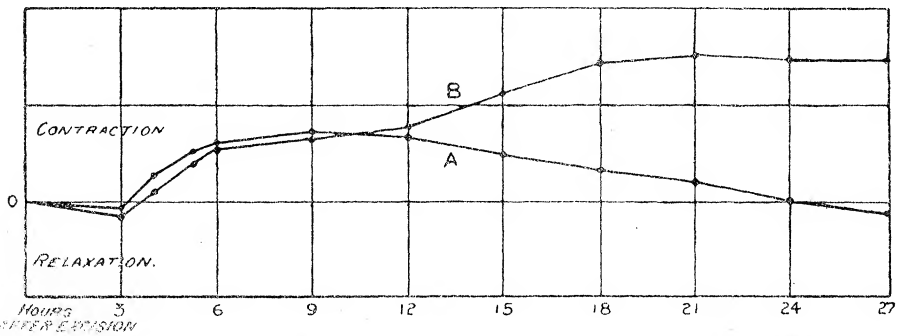


FIG. 4.—Changes in length of a pair of excised gastrocnemii, after fatigue. Ordinates and magnification as in fig. 3. Load, 3 grm. Temp. 16° C. A. Exposed to oxygen. B. Exposed to air. From the 'Journal of Physiology,' vol. 28, p. 479 (1902).

Now lactic acid itself is the most obvious determining cause of the signs both of fatigue and of the stiffening of rigor mortis. Its artificial application to muscle can mimic the signs of both (1). And, indeed, it had often been suggested from observations in the whole animal that lactic acid was a product of activity whose expulsion was effected by burning to carbon dioxide and water. One sign of the presence of lactic acid in fatigued muscle is to be found in a characteristic change of the osmotic properties of the muscle, and it was further shown at Cambridge that immersion of a fatigued muscle in oxygen restored the osmotic properties to those of resting muscle (4).

All these results pointed irresistibly to the conclusion that lactic acid

produced by muscle contraction or upon dying is oxidisable, or in some way removable by oxygen, with an accompanying production of carbon dioxide. The next step was to obtain direct evidence of the changes undergone by lactic acid in muscle. Lactic acid outside muscle, in the circulation for instance, is not directly oxidisable at physiological temperatures.

Lactic Acid in Muscle.

It is a remarkable fact that up to less than ten years ago we had little or no knowledge of the most elementary relations of this acid to the physiology of muscle. Production of free acid appears to be an almost universal sign of the activity of any living cell, and a sign also of the processes leading to death; but even in the conspicuous case of muscle nothing was known certainly with regard to the conditions of lactic acid production, save the fact of its happening. This is another striking instance of the slenderness of the foundation upon which the inogen hypothesis had been erected, and with it, as we have seen, almost the whole structure of prevalent ideas with regard to the general nature of the processes of metabolism.

The inherent difficulty besetting the chemical examination of muscle lies, of course, in the fact that the necessary processes for extraction of the constituents cause in the moment of their application profound chemical change. It will not be appropriate here to explain in detail the chief fallacies underlying the methods which had formerly been used. It may be said, however, that up to a few years ago there was hardly any single statement made with regard to the conditions of lactic acid appearance in muscle which was not both supported and contradicted by rival sets of observers respectively.

In our own work (5), of which we propose to give very shortly the chief results, we found that the disturbing influences introduced by the mechanical and chemical operations necessary to the process of investigation, could be reduced to a minimum if throughout the whole of their performance the muscle was maintained at a temperature close to the freezing point. Completely resting muscle examined in this way in the cold, when the cold is maintained until the extracting processes are complete and the muscle killed, gives only the smallest traces of lactic acid, and these traces must be attributed to the unavoidable minimum of manipulation before the low temperature is reached. Resting muscle, that is to say, may be regarded as muscle containing at most only traces of free lactic acid.

In order to determine the lactic acid production associated with any particular muscular condition, whether of fatigue or of spontaneous resting change, the processes of examination were carried out again only when the

temperature had been brought to the freezing point, by which the *status in quo* could be maintained.

The first qualitative estimations that were undertaken showed at the first attempt that fatigued muscle contained more lactic acid than resting muscle, and that fatigued muscle after resting in an oxygen atmosphere subsequently contained less lactic acid—a result which was confidently expected in view of the experiments which have been described already.

Attempts were then made to improve the technical methods for the accurate quantitative estimation of small quantities of lactic acid under the required conditions. In the end, and after trial of alternatives, resort was had to the old method of estimation by weight of the zinc salt obtained from the dextro-rotatory acid which muscle yields, and in the details of this method certain improvements were effected.

The chief facts relating to the production of lactic acid in the muscle substance, as these have been determined by our estimations, may be shortly stated.

Mechanical injury, like that of chopping up the muscle, produces a rapid increase of lactic acid. This rate of production is accelerated by rise of temperature, and is brought to a standstill at the freezing point.

Isolated undamaged muscle left at rest in air at ordinary temperatures continues to yield lactic acid, so that the total acidity progressively and steadily increases for many hours. Outward signs of this acid production are found in visible physical changes of the muscle, as shortening, stiffening, and loss of translucency, and it is accompanied by a corresponding yield of carbon dioxide previously held in the muscle, but now expelled by the increasing acid.

As the temperature increases, this spontaneous yield of lactic acid is accelerated. Between 35° C. and 40° C. it is very rapid and reaches a maximum almost instantaneously. This is the "acid maximum" formerly described by Ranke. Nevertheless, if the muscle be rapidly scalded, the source of lactic acid is "fixed," and little or no acid production takes place, as du Bois Reymond formerly showed.

If the muscle be left at room temperatures in nitrogen or other anaërobic atmospheres, it yields lactic acid at a uniform rate determined by the temperature, and so approaches and finally reaches the acid maximum. It reaches it, however, faster than it does in air at the same temperature. In oxygen, on the other hand, it is found to accumulate no lactic acid at all during many hours or indeed during days at room temperatures.

Upon stimulation an increase of lactic acid is found. This had been known of course from du Bois Reymond's time, but the fact had been repeatedly

denied, and the actual quantitative evidence supporting it had been wholly unsatisfactory.

If a muscle so fatigued, and containing lactic acid, be now left at rest in an

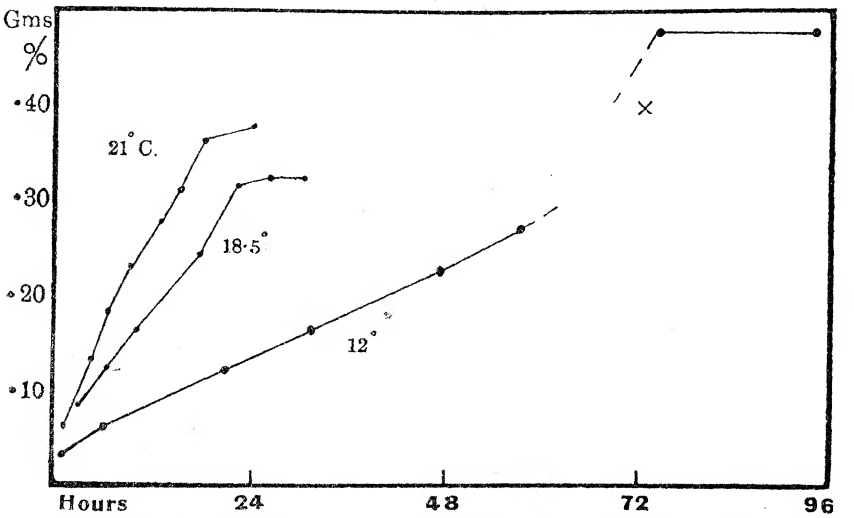


Fig. 5.—The course of lactic acid production in an atmosphere of hydrogen at 12° C. (Figs. 5, 6, and 7 from the 'Journal of Physiology,' vol 35, p. 273 (1907).)

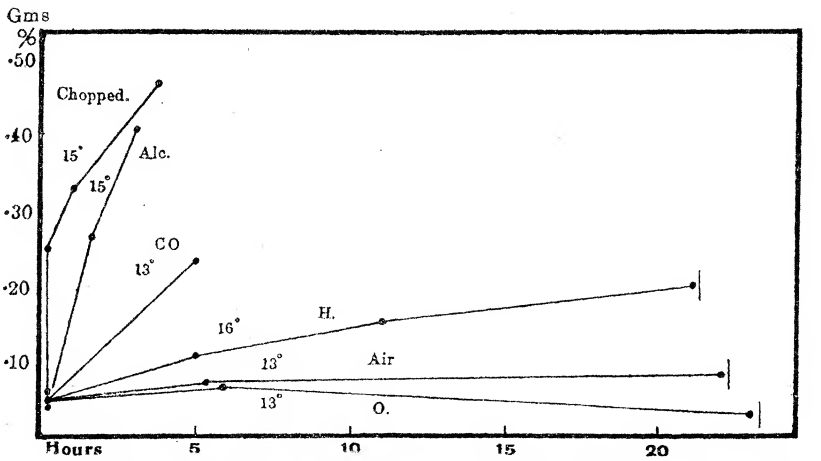


Fig. 6.—The course of lactic acid production occurring during survival periods in oxygen, air, hydrogen, and coal gas (CO) respectively. The two uppermost curves are introduced for comparison of those for chopped muscle and for alcohol immersion.

oxygen atmosphere, a notable decrease of lactic acid occurs. The acid is diminished quickly at first and later more slowly. It disappears, just as we saw earlier that fatigue or the stiffening of early rigor mortis disappears, when

the fatigued or dying muscle is placed in oxygen. With a rise of temperature above 30°, however, the accelerated spontaneous production of lactic acid overcomes this oxidative removal; the muscle enters into heat rigor and develops the acid maximum in spite of the presence of oxygen (fig. 7).

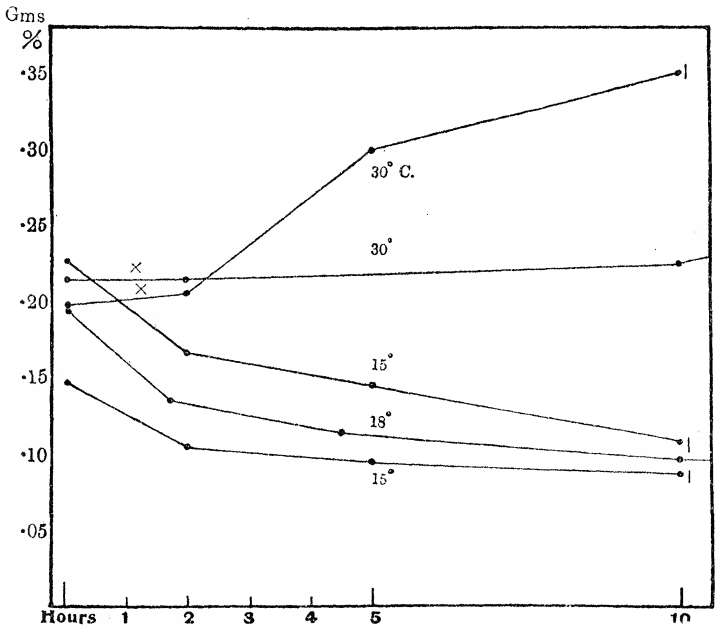


FIG. 7.—Lactic acid production and loss, in atmosphere of oxygen at different temperatures. Fatigued muscles were used for all. At 30° C. gain in lactic acid is shown: at 15–18° C. the course of loss is followed. × loss of excitability.

Severe mechanical injury, moreover, produces an acid yield which is unbalanced by oxidative removal, and muscle chopped in pieces or ground up with sand in the presence of oxygen very rapidly reaches the acid maximum, and reaches it apparently as rapidly as if oxygen were absent. It seems that the normal architecture of the muscle is a necessary condition for the oxidative process of removal.

These results as here stated very shortly, when set side by side with the facts of the carbon dioxide output already given, show clearly again that oxygen does in fact enter the living substance of muscle for the purposes of an immediate oxidation, and not as a preparer or builder up of material ready for explosion. We are now therefore in a position to distinguish more clearly those chemical events in muscle which are anaërobic and independent of oxygen, from those, on the other hand, in which oxygen plays a part.

Plainly the act of contraction and the process of rigor, each with its accompaniment of lactic acid formation, are anaërobic functions. Neither of them, it

is to be emphasised again, is associated with any fresh yield of carbon dioxide, except such as is directly due to expulsion, by the lactic acid, of previously formed carbon dioxide loosely combined in the muscle.

In the oxygen atmosphere, however, we have a removal of acid, a simultaneous yield of newly-formed carbon dioxide, with a restoration of the *status in quo ante* and of the previous "potential" of the muscle. Carbon dioxide production in an atmosphere of oxygen is a sign and a measure of an immediate contemporary combustion.

The Heat Production of Muscle.

These results, it must be pointed out, are the results of the chemical study of an integrated series of contractions in a muscle. The present limitations of chemical method do not allow us to measure and follow the time relations of the relatively minute changes which accompany and succeed each single act of contraction.

During recent years, however, Mr. A. V. Hill has conducted, at Cambridge, a long series of investigations into the heat production of muscle by means of the most refined thermo-electric methods. Of this work we must not pretend here to give any adequate account, but we must notice in general that, following up the results of our own experimental and chemical work, and using the same general experimental methods of analysis of the conditions of fatigue, of rigor (whether inflicted by heat or by chloroform), of recovery in oxygen, and so on, Mr. Hill has obtained a valuable series of parallel observations of heat production which have fundamental importance for the theory of muscular metabolism. By ingenious modifications of the thermopile and with a highly sensitive galvanometer Mr. Hill has been able to record the temperature changes associated with a single act of contraction, and so by exposing the muscle either to nitrogen or to oxygen the anaërobic heat production can be distinguished from the aërobic.

He finds that if the muscle contracts after being an hour or more in nitrogen, the heat production observed as the accompaniment of contraction does not continue beyond it (12). In oxygen, however, the heat production of contraction is continued for long periods after the mechanical event is over. The amount of heat liberated during the recovery process in oxygen he found to be at least as great as that due to the anaërobic act of contraction itself.

We must not now stay to speak of the many other general respects in which Mr. Hill's work has confirmed and further illuminated our own observations made on the chemical side.

Before passing, however, to some general considerations, we would draw attention to the fact that for important reasons of technique (which we must

not now discuss) all these experimental results at Cambridge have been gained by the use of muscle isolated from a cold-blooded animal. We have already, however, sufficient assurance that in all essentials the results can be taken to apply equally, *mutatis mutandis*, to the case of warm-blooded mammalian muscle (16). Verzar, at Cambridge (9), Winterstein and others abroad, have also confirmed our results with amphibian muscle by showing them again in the case of mammalian muscle.

The considerations which have so far been brought forward seem to lead to a conclusion from which there is no escape. The special processes which, when they occur within a muscle fibre, culminate in a contraction, make no call upon an oxygen supply; they proceed anaërobically. The oxidations which are always associated with muscular activity are separated in time from that moment in which mechanical energy is liberated. They occur immediately afterwards, and are concerned not with the induction of the mechanical act, but with a restoration to the *status quo ante*. They are concerned not with stimulation but with recuperation.

Our problem, then, is to find a full description for each of these two phases of change within the muscle, the anaërobic leading to contraction, the oxidative resulting in recovery.

We are concerned in this Lecture in the main with the respiratory or oxidative phenomena, but in dealing with these we must, of course, have regard to the muscular act as a whole. Here we would beg in advance your indulgent consideration; the main problems before us, as we have urged already, lie at the centre of what knowledge we have of the processes of cell life. They lie close at every point along their borders to other great fields of physiological enquiry. Their full, or, indeed, their adequate, discussion should involve reference to manifold considerations in regard to the facts of general metabolism, to questions of chemical energy and its transformations, to the phenomena of electrophysiology, and to the intricate problems of colloidal molecular physics. In the brief scope of one lecture, we can only attempt to point to the considerations which arise more immediately from the experimental results we have given.

In the first place, for the sake of greater clearness, it will be well to point at once to the provisional conception we adopt of the part played in muscular motion by lactic acid itself. So far from this being regarded as a toxic product to be eliminated as rapidly as possible, there is abundant reason supplied by many lines of converging evidence for seeing in lactic acid an essential agent in the machinery of contraction itself. The development of acid, with free H-ions, in the neighbourhood of colloidal fibrils gives the condition for contraction, whether by increasing the

molecular tension along longitudinal surfaces, or whether by the process of imbibition, causing a resultant increase of tension in the fibre. A catgut fibre in water will contract if its temperature be raised, as Engelmann showed here in his Croonian Lecture of 1895, and it will contract if acid be brought to it, relaxing again on its removal. We know that artificial application of lactic acid to muscle causes contraction, reversible by removal, as one of us showed long ago (1), and we have noticed to-day the shortening produced in muscle as lactic acid accumulates after fatigue and the lengthening which follows its removal by oxygen (Fig. 4). Engelmann thought of muscle as a heat engine, but we know now that, apart from other theoretical objections to that view, the heat production of contraction may take place after the mechanical contraction is over, and, in physiological conditions with oxygen present, the greatest heat production is always subsequent in time to the contraction. But, in spite of this dislocation in time, there is, as Hill has shown (7), a constant ratio between the new tension set up in the contractile elements and the heat of the contraction. The heat of the contraction, however, will be proportionate to the chemical reaction yielding it, so that we may say that there is a constant ratio between the increased fibrillar tension and the new chemical condition causing it, and regard the muscle as a chemical instead of as a heat engine; this, indeed, very many other considerations, into which time now forbids us to enter, force us to believe. Such a chemical event, proportionate to heat production, and also to the new tensile stress, would be the appearance, close to the muscle-fibrils, of the H-ions of lactic acid as this arises from some forerunner. The new condition of elastic state of the fibrils will give contraction if the mechanical conditions allow it, and work will be done according to the opposition given during the contraction. The work actually done, however, will bear variable and quite accidental relations to the heat production, as we have long known that it does, and the mechanical efficiency of the machine will vary accordingly with the conditions. The removal of lactic acid under the influence of oxygen will give relaxation, as the original state of tension in the fibril is restored.

To complete the image before us, we have still to consider the nature and the results of this oxidative removal of the lactic acid, hoping to reconcile in hypothesis the ascertained facts with regard to energy exchanges and chemical events.

On the simplest view, we might picture the muscle fibre as endowed initially with a supply of a substance (probably derived from carbohydrate) capable of yielding lactic acid by a non-oxidative molecular rupture. This rupture is exothermic, and heat is yielded proportionately to the acid

formed, and to the new surface tension or elastic tension imposed on the fibril by the acid ions. If the lactic acid be allowed by repetitions of the process to accumulate, fatigue phenomena are produced, and on this view fatigue is the expression, not of an exhaustion of energy supply, but only of a clogging of the machine. With a normal oxygen supply, however, the lactic acid is promptly removed after each contraction, and each successive stimulus, with its associated breakdown, is followed by a normal contraction. The removal of the lactic acid might be thought of as a direct oxidation—in the presence of oxidases—and it might be supposed to be burnt, so to speak, to waste, when the energy liberated by its combustion would supply nothing to the mechanical energy of contraction.

But this simplest view we are driven at once to forgo, and perhaps not unwillingly, since it would be unwelcome to believe that a body of such high energy value as lactic acid can be only a waste product yielding nothing in its discharge except the indirect benefits of heat production unconnected with the muscle machinery. Direct observation assures us, however, that of the energy of combustion of the lactic acid part at least remains in the muscle, for the work at Cambridge, both of Hill and more lately of Parnas (20), though they differ in other vital respects, concurs in this, that the energy leaving the muscle as heat in the oxidative removal of lactic acid is less than that calculated for the combustion in the oxygen used up. Some of the energy of that combustion is restored in some shape and to some degree at least to the muscle system.

On this account the general trend of opinion based upon the recent work we have described has returned to an old conception tentatively offered by Hermann, who suggested that in the building up of the inogen molecule there might enter again, with oxygen, part of the lactic acid from the previous contraction, there to be arranged in the explosive complex from which at the next contraction lactic acid and carbon dioxide should emerge.

There are grave theoretical difficulties associated with the conception of an "inogen" capable of rapid breakdown and rebuilding, if it is to be formed, as we have shown it must be, without the inclusion of oxygen. Yet the idea that the lactic acid, instead of being burnt away, may be actually restored into its former position in the molecule of its precursor, by the energy of a combustion of some other material in the oxidative recovery, has gained ground lately, and partly on account of an observation of our own. In our studies of the lactic acid of frog's muscle we made out the following facts. Suppose for a given set of similar muscles the "maximum" lactic acid production, as induced by heat rigor, be determined. Suppose, further, that another comparable set of muscles be stimulated to fatigue, then allowed to

recover in oxygen, again stimulated, and again submitted to oxygen, these alternate processes being many times repeated. It is clear that, since lactic acid is produced during each period of stimulation, and removed during each period of recovery in oxygen, heavy drafts must be made upon the precursor of the acid in any experiment such as that described. Nevertheless, a set of muscles, after having undergone such treatment, give, when thrown into heat rigor, exactly the same maximum yield of lactic acid as a set of perfectly fresh muscles (fig. 8).

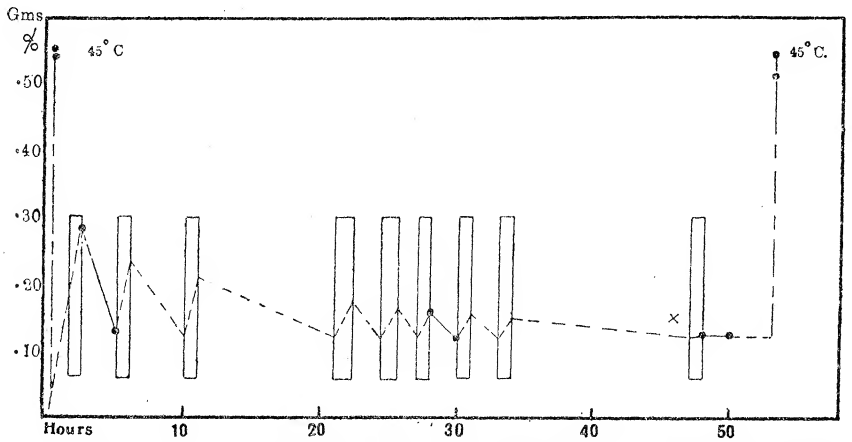


Fig. 8.—The relation of the heat-rigor lactic acid "maximum" to the survival history of muscle. Four estimations of lactic acid due to heat rigor are shown, two at the beginning, in the case of resting muscles, two at the 53rd hour, in the case of inexcitable muscles, which had gone through nine periods of severe stimulation alternated with periods of rest in an oxygen atmosphere. The enclosed areas represent time periods (drawn proportionate to abscissæ) of stimulation by strong interrupted shocks. × loss of excitability. Temperature 15° C. Continuous line shows course of acid loss as actually determined by estimation. Dotted line shows the presumed course of acid loss and gain during other alternate periods. (From the 'Journal of Physiology,' vol. 35, p. 293 (1907).)

In discussing these results, we suggested as a possible explanation (though we discussed alternative possibilities) that lactic acid is not oxidised during the recovery of the muscle, but is rebuilt into the complex from which it was derived, at the expense of energy derived from the oxidation of something else. The formation of this unstable complex would then be the basis for that restoration of potential which we have just been considering.

This view has been widely adopted, but we ourselves are now disposed to doubt it. It was shown at Frankfort by Kondo,* in Embden's laboratory, that the formation of lactic acid in expressed muscle-juice is due to a

* 'Biochemische Zeitschrift,' vol. 45, p. 63 (1912).

chemical reaction which is inhibited by acid, and is therefore self-controlled. It was suggested, therefore, that the maximum production in heat rigor does not represent the total exhaustion of the lactic acid precursor, but rather the normal cessation of a reaction at a critical concentration of hydrogen ions. So long as a sufficiency of the precursor is present, therefore, at the end of an experiment such as that described above, there is no difficulty about the attainment of a similar "maximum" by muscles restored after fatigue, and by fresh muscles, respectively. If this be so, our experiments gave no proof of a reconstruction of the lactic acid into something else. The experiments of Embden were not wholly satisfactory, because in muscle-juice lactic acid has so nearly reached a maximum, as the result of the expression of the juice, that the amount of change to be observed is but small. But, at our suggestion, Mr. Winfield has recently carried out some experiments at Cambridge upon intact muscles placed in Ringer's solution which fully confirm the fact that acid production in muscle depends upon a self-limiting reaction which ceases when a certain grade of acidity is reached. We are inclined, therefore, to doubt if there be any evidence on these direct lines that the lactic acid in recovery processes is rebuilt into a precursor in such a way as to restore the former level of the source of acid supply.

On this question of the possible restoration of lactic acid to its former molecular position, Hill has discussed some indirect evidence derived from his own thermodynamic studies. Peters found at Cambridge (14), by an adaptation of Hill's thermo-electric methods, that the heat production of chloroform rigor was equal to the sum of the two stages of heat production in similar muscles, in the first stage stimulated to give a partial lactic acid yield, and in the second stage killed with chloroform to give full rigor and the acid maximum. This observation in itself is interesting as an added proof that the heat production, like the lactic acid production, is derived from the same source whether in contraction or in rigor. Taking this total heat production found by Peters, together with our own lactic acid estimations for the same conditions, Hill argues that there is a heat production of 450 calories for each gramme of lactic acid formed. But his own results showed that the heat of oxidative removal of the acid was approximately equal to the heat of production, so that the oxidative removal of 1 grm. should yield about 450 calories. But the combustion of 1 grm. of lactic acid yields about 3700 calories. Hill urges accordingly that the indication of our experiment just quoted (fig. 8) should be accepted, and the lactic acid regarded as being replaced in its former position in the muscle during the oxidative recovery, the energy for that restoration of potential being derived from the combustion of some other constituent (*e.g.* carbohydrate)

in the muscle. The lactic acid on that view would be "part of the machinery and not part of the fuel," to use a familiar Cambridge phrase.

As opposed to this argument advanced by Hill we have, however, some more direct evidence supplied by Parnas, whose work at Cambridge with us was interrupted by the war, but has been continued in Germany (22). He compared the oxygen consumed by fresh resting and by fatigued muscles respectively, and thus determined the excess which was due to the process of recovery. From this he calculated the total heat which would correspond, in combustion, with the oxygen consumption observed. He then determined the actual heat production of similar muscles during the recovery process in oxygen after fatigue. This he found to be only half of that indicated by the oxygen consumption, and concluded that this retained energy was stored during the restoration of potential to the muscle. He suggests that the lactic acid is in part burned away, the heat supplying energy for restoring what he describes as the physico-chemical state of the resting muscle. Unfortunately, these experiments have not yet been described in detail, and it is therefore difficult to appraise their value. The technique used in the estimation of the heat given out by muscles is based upon the methods developed by A. V. Hill, and would appear to be satisfactory. One criticism occurs to us, however. The actual amount of energy presumed to be stored as potential in the fibres has relatively a very small caloric value: thus, during the whole process of complete recovery from full fatigue, Parnas found that only about two gramme-calories were stored per gramme of muscle. We are by no means clear that any correction was made for the latent heat of evaporation of water from the surface of the muscle, and this we conceive might greatly affect the quantitative value of such a measurement.

But it must be admitted that when fresh unfatigued muscles were used, there was apparently a close correspondence between the heat calculated from their oxygen consumption and that actually given out, which could hardly have been the case if surface evaporation had been occurring.

For the present we feel bound to conclude, upon the evidence as to heat production advanced both by Hill and by Parnas, that while the lactic acid produced during contraction is itself the material which is then immediately oxidised with a yield of carbon dioxide, part at least of the heat of combustion of lactic acid is stored in potential form in the muscle as it returns to the resting state.

We shall now endeavour to justify our belief that lactic acid itself is, as a matter of fact, the material actually oxidised in muscle.

Among those who, during recent years, have investigated, or considered with expert knowledge, the intermediary processes of metabolism, not from

the narrower standpoint of muscular activity alone, but in connection with the animal body as a whole, there has been almost complete unanimity in believing that lactic acid is an intermediary product on the main lines of carbohydrate metabolism. There is cogent evidence for this view, though it would, of course, be out of place for us to discuss it here. But it is the muscle in which by far the greater part of the total metabolism of the body takes place, and if, in muscle, lactic acid must be supposed in normal circumstances to appear only momentarily, and then, instead of following further steps towards the end-products of metabolism, to suffer instead a return to its source, it would be difficult to reconcile its history in muscle with what is believed concerning its importance in general metabolism.

It is significant in this connection to find, as we do (23), that the pancreas which exerts so important an influence upon the processes of general carbohydrate metabolism, exercises a direct control over the formation of lactic acid in muscle.

If it be not the lactic acid which is burnt, we must seek alternative fuel for the undoubted combustion which occurs. Hill suggested that carbohydrate as such was the fuel, giving energy for the restoration of the lactic acid to its former position. But Parnas and Wagner (24) have supplied definite evidence that carbohydrate, while it disappears from the muscle during the anaërobic processes in which lactic acid appears, remains unchanged in amount during the oxidative recovery.

Winfield at Cambridge has shown that fats, moreover, are not oxidised in the excised muscle (19), and we have good reasons, finally, to believe that in normal circumstances protein material is not burnt. But if neither protein, fat nor carbohydrate is the fuel we seek, what then is the material which undergoes oxidation? The only justification for doubting that it is lactic acid, the one substance which obviously accumulates in the absence of oxygen, and disappears in its presence, was the suggestion derived partly by tradition from the teaching of Hermann, and partly from the supposed evidence, already criticised, that the lactic acid disappears because from it is reconstituted the unstable substance, the "inogen," imagined to be the immediate source of the contractile energy. Apart from the absence of direct evidence in its favour, there are grave difficulties associated with the conception of an "inogen" capable of rapid breakdown and reconstruction, if we now abandon, as we have shown earlier that we must, the idea of the inclusion within it of oxygen. It seems almost impossible to conceive of an organic substance derived from lactic acid, and not containing "intramolecular" oxygen with unstable attachments, which could, by a non-oxidative rupture of its molecule, yield the energy required for contraction,

particularly if this, as Parnas's experiments seem to show, amounts to not less than half the oxidative energy of lactic acid. In our opinion the conception of a chemical "inogen" of any kind is false, and fated to disappear.

The high potential energy required for the rapid act of contraction may be stored, however, in some other form. The potential, which is lost upon contraction and restored by subsequent oxidation, may reside, not in an unstable chemical substance, but in a particular condition of a physico-chemical system. Here we may return to the image we previously depicted of the muscle machinery in our provisional hypothesis.

In a system of colloidal fibrils, or of longitudinal surfaces, into relation with which H-ions of lactic acid lie ready to be brought, we have a potential of energy which may be discharged as work, with or without heat, on the development of a new state of tension in the fibrils, whether tension due to inhibition or to added surface tension along the longitudinal surfaces. The observed heat production of anaërobic contraction may be in part due to the exothermic molecular change which yields the free acid from its precursor, and in part due to the resultant change in colloidal surfaces or substances upon the delivery to them of the acid ions.

Upon recovery by oxidative removal of the lactic acid, the energy of combustion is discharged in part as heat and in part (and what fractional part that is we have seen to be at present uncertain) returned to the muscle in the restoration of the initial potential. In this restoration will be involved the separation of the acid ions from the colloidal fibrils, by which the condition will be given for the return of the fibrils to their former tension—the tension, that is to say, of the muscle in the state of relaxation and rest, and possessed of the potential inherent in them.

We have been speaking so far of changes of potential in connection with the contractile act. As regards the actual main reservoir of energy, it is clear that this must be contained within the muscle itself, because most of our data have been obtained from excised muscle. That this main reservoir of enérgy is to be sought in the carbohydrate stores is, we believe, quite certain. The question arises, Are we to assume that carbohydrate must first be converted into a substance of higher chemical potential before it can serve as a contributory source of contractile energy by its breakdown to lactic acid? The small energy change which that breakdown involves has been thought by some to make this assumption necessary. It is just this assumption, however, that the conception of a change in the physico-chemical system of colloid fibrils, as the vehicle of a rise of potential, makes unnecessary. The contractile act may call, not only upon the chemical energy liberated when sugar becomes lactic acid, but also, and

perhaps to a greater degree, upon the energy derived from the oxidation of the lactic acid, residing in the physico-chemical system of the muscle, which was produced during the previous contraction.

If our picture of events is the true one, and if the machinery of contraction is of the kind we have suggested, then carbohydrate metabolism in muscle takes on an aspect of peculiar interest.

We have already recalled the evidence gained from studies in general metabolism, made without special reference to muscle, and have shown that it points clearly to the conclusion that sugar does not suffer oxidation as such, but only after it has first, at an early moment in its metabolic progress, passed through the stage of lactic acid.

But in the muscles, which after all form the chief seat of metabolism, the acid intermediary product appears, if we are right, at such a stage and place as to have more than a purely chemical significance. It marks, on the one hand, an obligatory stage in a particular set of successive chemical reactions; but, on the other hand, it has here its special *rôle* to play in connection with the muscle machinery. In the evolution of muscle it would appear that advantage, so to speak, has been taken of this acid phase in carbohydrate degradation, and that by appropriate arrangement of the cell elements the lactic acid, before it leaves the tissue in its final combustion, is assigned the particular position in which it can induce those tension changes upon which all the wonders of animal movement depend.

In concluding, we would endeavour to convey in brief terms our reasons for thinking that the particular standpoint thus taken is one which makes for simplicity and clearness in our views concerning muscle, and perhaps in more besides.

Underlying all views concerning the source of contractile energy, there has persisted till recently, almost as a tradition in physiology, the obstinate assumption that this energy must necessarily be sought in an unstable chemical substance of complex and unknown constitution—perhaps in the protoplasmic molecule itself, perhaps in an "inogen" vaguely to be distinguished from the protoplasm, perhaps only in some compound of a more definite sort in which carbohydrate matter finds itself transformed and endowed with a higher chemical potential. It must surely bring a gain to the clearness and simplicity of our conceptions, and bring encouragement also to the experimentalist, if such an assumption with its many attendant difficulties, to some of which we have alluded, should prove unnecessary. We believe it to be so. With an understanding that the relatively permanent physico-chemical system of the muscle can, without itself

undergoing chemical modification, carry changes of potential as a result of changes in its physical configuration, it becomes easier for us to realise that the food-stuffs, or at least that sugar, may be the direct source of the contractile energy. Placed in the right locality within the muscle, sugar, by a non-oxidative yield of acid at the right moment, and by a subsequent oxidation of this at another right moment, can yield its total energy in a manner exactly suited to serve the peculiar machinery in which, so to speak, it finds itself.

The actual chemical events which underlie the obvious manifestations of change in muscle—the contraction, the exhibition of fatigue, the recovery—we might then regard as relatively simple. We find similar indications in all progressive departments of biochemistry. The chemical events are not in themselves necessarily complex or obscure; the complexity is found in the conditions under which they occur. The difficulties of the biological enquirer arise from the fact that he has, for the most part, to accept these conditions as given. It is usually open to the physicist or pure chemist to control and simplify the conditions of his experimental work, or wisely to avoid regions of complexity until collateral progress has made them simple. In biology the complexities of the conditions are in the essence of the phenomena, and the experimentalist, when he tries to simplify them, is even viewed with suspicion. Thus even the operation of excising a muscle before studying its chemistry has been regarded with some prejudice, though in this case we think we may fairly claim that the progress made in the long series of enquiries we have discussed, has illustrated the fact that the biologist is after all not wholly shackled by the necessity of putting all his questions to the intact animal.

The description of muscle activity we have attempted to give remains, it is true, imperfect; indeed, we hardly yet have knowledge enough to guess how imperfect it is. But recent studies have had at least the result of confirming our own faith in the powers of experiment to bring improvement of knowledge, and we venture to believe that they have already indicated hopeful lines for further experimental work.

The following publications, to which reference has been made in the Lecture, are based upon researches which have been carried out in the Physiological Laboratory at Cambridge:—

(1) W. M. Fletcher, "The Survival Respiration of Muscle," 'Journal of Physiology,' vol. 23, p. 10 (1898).

(2) W. M. Fletcher, "The Influence of Oxygen upon the Survival Respiration of Muscle," *ibid.*, vol. 28, p. 354 (1902).

(3) W. M. Fletcher, "The Relation of Oxygen to the Survival Metabolism of Muscle," *ibid.*, vol. 28, p. 474 (1902).

(4) W. M. Fletcher, "The Osmotic Properties of Muscle and their Modifications in Fatigue and Rigor," *ibid.*, vol. 30, p. 414 (1904).

(5) W. M. Fletcher and F. G. Hopkins, "Lactic Acid in Amphibian Muscle," *ibid.*, vol. 35, p. 247 (1907).

(6) A. V. Hill, "The Heat produced in Contracture and Muscular Tone," *ibid.*, vol. 40 p. 389 (1910).

(7) A. V. Hill, "The Position occupied by the Production of Heat, in the Chain of Processes constituting a Muscular Contraction," *ibid.*, vol. 42, p. 1 (1911).

(8) W. M. Fletcher, "On the Alleged Formation of Lactic Acid in Muscle during Autolysis and in Post-survival Periods," *ibid.*, vol. 43, p. 286 (1911).

(9) F. Verzár (Budapest), "The Gaseous Metabolism of Striated Muscle in Warm-blooded Animals," *ibid.*, vol. 44, p. 243 (1912).

(10) A. V. Hill, "The Heat-production of Surviving Amphibian Muscles, during Rest, Activity, and Rigor," *ibid.*, vol. 44, p. 466 (1912).

(11) G. R. Mines, "On the Summation of Contractions," *ibid.*, vol. 46, p. 1 (1913).

(12) A. V. Hill, "The Energy degraded in the Recovery Processes of Stimulated Muscle," *ibid.*, vol. 46, p. 28 (1913).

(13) A. V. Hill, "The Absolute Mechanical Efficiency of the Contraction of an Isolated Muscle," *ibid.*, vol. 46, p. 435 (1913).

(14) R. A. Peters, "The Heat Production of Fatigue and its Relation to the Production of Lactic Acid in Amphibian Muscle," *ibid.*, vol. 47, p. 243 (1913).

(15) A. V. Hill, "The Heat Production in Prolonged Contractions of an Isolated Frog's Muscle," *ibid.*, vol. 47, p. 305 (1913).

(16) W. M. Fletcher, "Lactic Acid Formation, Survival Respiration and Rigor Mortis in Mammalian Muscle," *ibid.*, vol. 47, p. 361 (1913).

(17) W. M. Fletcher and G. M. Brown, "The Carbon Dioxide Production of Heat Rigor in Muscle and the Theory of Intra-molecular Oxygen," *ibid.*, vol. 48, p. 177 (1914).

(18) Viktor Weizsäcker (Heidelberg), "Myothermic Experiments in Salt Solutions in relation to the Various Stages of a Muscular Contraction," *ibid.*, vol. 48, p. 396 (1914).

(19) G. Winfield, "The Fate of Fatty Acids in the Survival Processes of Muscle," *ibid.*, vol. 49, p. 171 (1915).

(20) Parnas (Vienna), "The Transformation of Energy in Muscle," *ibid.*, vol. 49, p. vii (1914).

(21) Viktor Weizsäcker (Heidelberg), "Neue Versuche zur Theorie der Muskelmaschine," 'Münchener Med. Wochenschrift,' vol. 62, p. 217 (1915).

(22) Parnas (Vienna), "Ueber das Wesen der Muskelerholung," 'Zentralblatt für Physiologie,' vol. 30, p. 1, April, 1915.

(23) F. G. Hopkins and G. Winfield, "The Influence of Pancreatic Extracts on the Production of Lactic Acid in Surviving Muscles," 'Proc. Physiol. Soc.,' October 16, 1915.

(24) Parnas and Wagner (Vienna), "Ueber den Kohlenhydratumsatz isolierter Amphibienmuskeln und über die Beziehungen zwischen Kohlenhydratschwund und Milchsäurebildung im Muskel," 'Biochemische Zeitschrift,' vol. 41, p. 389 (1914).